

Review on *Trypanosoma vivax*

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Abstract: Trypanosomosis is a protozoal disease caused by the genus *Trypanosoma* affecting human and animals mainly in sub-Saharan Africa and also in Latin America. *Trypanosoma vivax* (*T. vivax*) is transmitted cyclically by tsetse flies only found in Africa while the mechanical transmission of *T. vivax* is worldwide problem including tsetse free regions of Africa. The disease is prevalent in two main regions of Ethiopia, northwest and southwest regions. The northwest region is particularly affected by both tsetse and non-tsetse transmitted trypanosomosis and the dominant species of trypanosome is *T. vivax*. The molecular characterization of *T. vivax* from Brazil, Venezuela and West Africa (Nigeria), corroborating the West African origin of South American *T. vivax*, whereas a large genetic distance separated these isolates from the East African isolate (Kenya). The disease results in clinical syndromes such as anemia, emaciation and mortality. Anaemia appears with progressing parasitaemia resulting in a drop in packed cell volume (PCV). The ability of trypanosomes to change their surface-coat-antigen continuously leads to the exhaustion of the antibody production by the host leading to immunosuppression. *T. vivax* infections in West Africa are rapidly fatal compared to the East and Central Africa but there are exceptions to this rule. Occasional outbreaks of haemorrhagic *T. vivax* infections in Kenya are rapidly fatal. *T. vivax* infection can be diagnosed by clinical, parasitological, immunological and molecular methods. A pondered evaluation extrapolated for the total tsetse-infested lands values total loss, in terms of agricultural Gross Domestic Product, at US\$ 4.75 billion per year. Studies on *T. vivax* in Latin America show that their economic impact can be quite severe. Control of the disease should combine treatment of infected animals and vector control. Eventhough, trypanocidal drugs will continue to play an important role in the integrated control of trypanosomosis, the development of trypanosome resistance to trypanocides is a continuous threat to their sustainable use. The available information on the pathogenic difference, drug resistance problems and genetic composition of *T. vivax* in relation to geographical locations and in different species of hosts is not yet elucidated in Ethiopia and hence requires thorough investigations.

Key words: *Trypanosoma vivax* • Biology • Epidemiology • Economic Impact • Genetic Diversity

INTRODUCTION

Trypanosomosis is a worldwide disease caused by the species of the genus *Trypanosoma*, which affects humans, as well as domestic and wild animals. Trypanosomes are unicellular organisms (Phylum Protozoa) belonging to the genus *Trypanosoma*, the family Trypanosomatidae and the order Kinetoplastida. Species of trypanosomes infecting mammals fall into two distinct sections [1]: The first group is the Stercoraria (Subgenera *Schizotrypanum*, *Megatrypanum* and *Herpetosoma* that include species such as *T. cruzi*, *T. theileri* and *T. melophagium*) in which trypanosomes are

typically produced in the hindgut and are then passed on by contaminative transmission from the posterior. The second is the Salivaria (subgenera *Duttonella* (*T. vivax*), *Nannomonas* (*T. congolense*) and *Trypanozoon* (*T. brucei*), in which transmission occurs by the anterior station and is inoculative. Many species of trypanosomes occur as parasites in a wide variety of animals and some of these parasites have been spread by humans from Africa to other continents. For example, *T. vivax* had been introduced to the Americas, by the importation of West African cattle in the eighteenth and nineteenth centuries [2].

In Africa, *T. vivax* is a heteroxenous parasite present in regions populated by tsetse flies and the parasite develops in the proboscis of this invertebrate host. *Glossina* spp. is the only vector in which *T. vivax* is able to multiply and remain in the infective phase throughout the insect's life. Outside tsetse fly infested areas, the parasite is carried by other hematophagous flies where transmission is noncyclical. Thus, the parasites are mechanically transmitted across vertebrate hosts, with no growth or multiplication in the insects [1]. Of the three main species of tsetse-transmitted trypanosomes affecting ruminants in sub-Saharan Africa, only *T. vivax* has spread beyond the bounds imposed by its vector in Africa and established itself in South America.

Trypanosomosis is prevalent in two main regions of Ethiopia i.e. the northwest and the southwest regions [3]. In these regions tsetse transmitted animal trypanosomosis is a major constraint to utilization of the large land resources. The northwest region of Ethiopia is particularly affected by both tsetse and non-tsetse transmitted trypanosomosis [4-6]. Six species of trypanosomes are recorded in Ethiopia and the most important trypanosomes, in terms of economic loss in domestic livestock are: *T. congolense*, *T. vivax* and *T. brucei* [3].

Although morphometric studies, DNA fingerprinting and isoenzyme profiling suggest a West African origin for New World *T. vivax*, it differs from African *T. vivax* in the diversity of its surface antigens and its inability to infect tsetse and grow *in vitro* [7]. The molecular characterization of *T. vivax* from Brazil, Venezuela and West Africa (Nigeria), corroborating the West African origin of South American *T. vivax*, whereas a large genetic distance separated these isolates from the East African isolate (Kenya) analyzed [8].

The severity of the disease depends on the species and strain of trypanosomes involved. *T. vivax* infections are predominant in cattle in West Africa and rapidly fatal whilst *T. congolense* causes a chronic disease. In contrast, *T. vivax* may be commonly encountered in East and Central Africa but causes a mild disease in cattle in comparison to *T. congolense*. There are exceptions to this rule: for example, the haemorrhagic *T. vivax* infections that occasionally break out in Kenya are rapidly fatal [9]. Typical features of these infections include high, persistent parasitemia, fever and very pronounced anemia; also generalized visceral and mucosal hemorrhage, particularly in the gastrointestinal tract. In addition to the host-parasite interaction; the epidemiology of non tsetse-transmitted trypanosomosis (*T. vivax* and *T.*

evansi) is influenced by many factors. There may be seasonal outbreaks, where the populations of biting flies (Tabanids, stable flies, etc.) are influenced by important seasonal climatic differences.

According to Budd [10] African farmers spend 35 million US \$ per year on trypanocidal drugs to protect and cure their cattle. A pondered evaluation extrapolated for the total tsetse-infested lands values the total losses, in terms of agricultural Gross Domestic Product (AGDP), at US\$ 4.75 billion per year [11]. Studies on *T. vivax* and *T. evansi* in Latin America show that their economic impact can be quite severe. Even the inapparent losses of subclinical infections by *T. vivax* may be considerable and the same certainly applies to mechanically transmitted trypanosomosis in Africa; further economic studies are necessary in order to obtain reliable figures [8].

Control of the disease should combine restricted movement of diseased animals, treatment of *T. vivax* infected animals, epidemiological monitoring of the distribution and severity of the disease and vector control [12]. Drugs regarded as effective in the treatment of *T. vivax* infection include diminazene diaceturate [13] and isometamidium chloride [14, 15]. Trypanocidal drugs will continue to play an important role in the integrated control of trypanosomosis. However, the development of trypanosome resistance to trypanocides is a continuous threat to their sustainable use in the control of trypanosomosis [16]. Therefore this paper review the current state of knowledge about *T. vivax* and the problems faced in controlling the disease.

Specific objectives

- To assess the epidemiology and economic importance of *T. vivax*
- To assess scientific findings on the diagnostic and genetic diversity of *T. vivax*
- To show gaps on the disease problems of *T. vivax* for future research works

Classification of Trypanosomes: Trypanosomes are unicellular organisms of phylum *Protozoa* belonging to the order *Kinetoplastida*, the family *Trypanosomatidae* and the genus *Trypanosoma* and they are called as blood parasites or haemoparasites, which in the vertebrate host occur in the blood and tissue fluid. Trypanosomes are also known as haemoflagellates, as they progress actively by the movement of the thread-like filament called flagellum [1].

Species of trypanosomes infecting mammals fall into two distinct sections [1]: A) the Stercoraria (Subgenus *Schizotrypanum*, *Megatrypanum* and *Herpetosoma*) species such as *T. cruzi*, *T. theileri* and *T. melophagium* in which trypanosomes are typically multiplied in the hindgut and are then passed on by contaminative transmission from the posterior and B) the Salivaria (Subgenera *Duttonella*, *Nannomonas*, *Trypanozoon* and *Pycnomonas*), in which transmission occurs by the anterior station and is inoculative. Characteristically, Salivarian species by virtue of variant surface glycoprotein (VSG) genes are the only trypanosomes to exhibit antigenic variation. The increasing complexity of mammal-infecting trypanosomes has required their classification into subgenera, based on phylogenetic hypotheses. For example, for *T. vivax*, the subgenus *Duttonella* was introduced [17]. The systematic position of *Trypanosoma* among the protozoa and the revised classification of the mammalian trypanosomes according to Levine *et al.* [18] are as follows: Subkingdom: Protozoa, Phylum: Mastigophora, Class: Zoomastigophora, Order: Kinetoplastida, Family: Trypanosomatidae, Genus: *Trypanosoma*.

Many species of trypanosomes occur as parasites in a wide variety of animals. Some of these parasites have been spread by humans from Africa to other continents. For example, *T. vivax* had been introduced to the Americas, by the importation of West African cattle in the eighteenth and nineteenth centuries and *T. evansi* had already “escaped” from Africa far earlier by animal movements (In particular camels) between Africa and Asia [2].

Biology of *Trypanosoma (Duttonella) Vivax*: The principal species of the genus is *Trypanosoma vivax* found in ruminants and horses but not pigs, dogs, cats.

The subgenus also contains a morphologically similar but smaller species, *T. uniforme*, which were reported from a Giraffe from Tanzania found in most ruminants [19].

Synonymus: *T. caprae*, *T. angolense*, *T. cazalboui*, *T. bovis* and *T. viennei* [19].

Morphology: A sound knowledge of the basic features of the various trypanosomes enables the identification of each species and the exact cause of the disease. The morphology of trypanosomes as indicated in Figure 1 illustrates the fundamental features of a trypanosome (Trypomastigote) in a stained preparation made from the blood of an infected animal [2].

Various structures are suspended in the cytoplasm, the most prominent being the nucleus, which may be regarded as the command centre of the cell and which also plays a major part in reproduction. It contains DNA which is arranged in the form of genes and chromosomes; it represents the genetic information and is responsible for the manufacture of enzymes and other proteins of the cell. Trypanosomes are elongated and streamlined and tapered at both ends. The pellicle, the outer layer of the cytoplasm, is flexible enough to permit a degree of body movement, while retaining a definite shape. A flagellum arises near to the posterior end from a parabasal body and runs the length of the trypanosome; it may be continued beyond the anterior end of the body as a whip-like free flagellum and play in the movement pattern as seen with the microscope in fresh blood preparation particular for *T. vivax*, which moves rapidly forward between the blood cells, whereas other species often just wriggle around without showing much forward progress. Along the length of the body the pellicle and cytoplasm are pinched up into a thin sheet of tissue called the undulating membrane, through the outer margin of which runs the flagellum [2].

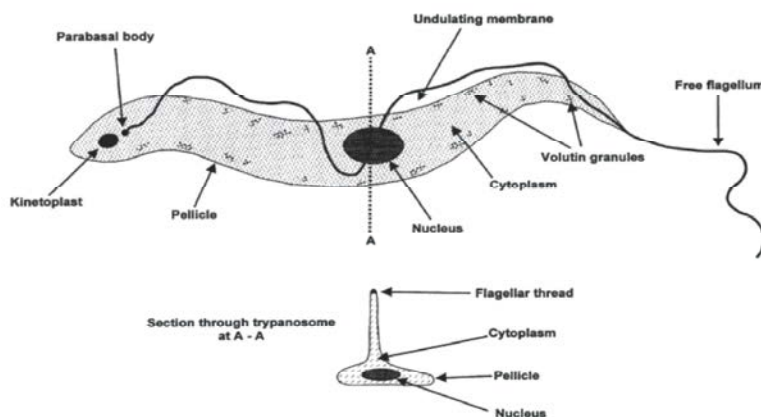


Fig. 1: Diagram of a trypanosome showing the fundamental morphological features, Source [2]

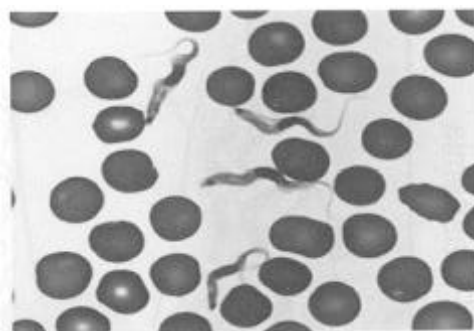


Fig. 2: Light micrograph of *Trypanosoma vivax* from mammalian blood, Source [2]

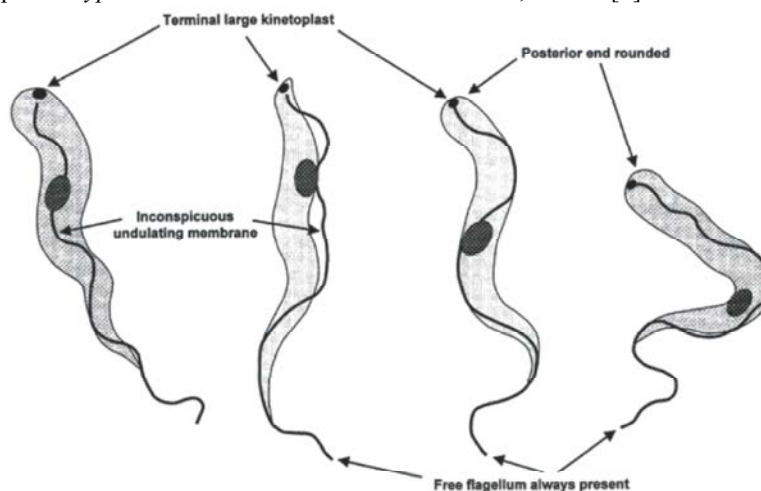


Fig. 3: *Trypanosoma vivax* blood stream forms, Source [2]

Among other basic morphological features, a distinct well-defined body, the kinetoplast, is seen near to the posterior end of the trypanosome and differs in size and position according to the species. It is adjacent to the parabasal body and so close to it that it cannot easily be seen separately with the light microscope. The kinetoplast has important functions in reproduction and metabolism and is probably essential for cyclical transmission by tsetse flies. It is sometimes absent in a proportion of trypanosomes, especially of some strains of *T. evansi*, a species which has lost its ability of being cyclically transmitted [2].

The length of *T. vivax* ranges from 21 μm to 25 μm which is distinctive from other Salivarian trypanosomes and more recent research on strains that naturally infect rodents and laboratory animals revealed the existence of longer, more granular bloodstream forms of *T. vivax* with a clearly subterminal kinetoplast [20]. The main characteristics of *Duttonella* bloodstream forms are large terminal kinetoplasts situated at a rounded posterior extremity, a medium developed undulating membrane and a free flagellum (Figure 2 and 3). A more slender form is sometimes seen, which possesses a more pointed

posterior extremity and has been thought to cause a more severe form of the disease. Such forms are commonly seen when *T. vivax* is dividing rapidly in the blood and it has also been reported that *T. vivax* in Latin America is more slender than the typical African parasite. *Trypanosoma uniforme*, small trypanosomes (From 12 to 20 μm), is otherwise similar to *T. vivax* [2].

In Africa, bloodstream forms of *Duttonella* trypanosomes exhibit a certain degree of dimorphism. These include club-shaped forms with rounded bodies that are swollen posteriorly and taper abruptly toward the anterior end and slender forms whose posterior end is also rounded, though not broader than the rest of the body, but tapers gradually toward the anterior end. Biometric analysis of several samples of *T. vivax* showed that body length ranges from 18 μm to 31 μm (Including the 3 to 6 μm -long free flagellum), with mean lengths ranging from 21 μm to 25.4 μm [1]. When cultivated in calves or sheep, they sometimes appear pleomorphic.

Life Cycle and Transmission: The life cycle has two phases (Figure 4), one in the insect vector and one in the mammalian host [1]. The development of *T. vivax* in

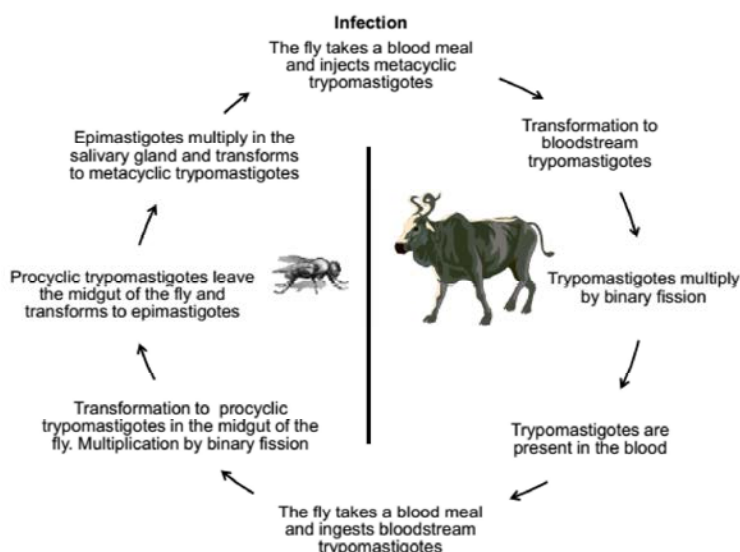


Fig. 4: The main phases in the life cycle of the trypanosome, both in the intermediate host (tsetse fly) and in the mammalian host, Source [21]

Glossina species is confined entirely to proboscis. In vertebrates, salivarian trypanosomes multiply in a continuous manner as trypomastigotes [17]. The infective metacyclic trypanosomes undergo development and multiplication at the site of infection where a swelling or chancre may be detected in the skin and finally the mature blood trypanosomes (Trypomastigotes) are released via lymph vessels and lymph nodes into the blood circulation. Reproduction in the mammalian host occurs through a process of binary division. Blood stream forms (trypomastigotes) ingested by the fly undergo considerable changes, in morphology as well as in their metabolism. They change into long slender forms called epimastigotes, which multiply and finally give rise to the infective metacyclic trypanosomes.

In Africa, *T. vivax* is a heteroxenous parasite in an area where tsetse flies are present. The parasite develops in the proboscis of this invertebrate host, where trypomastigotes evolve to epimastigotes. This is a crucial phase, as it leads to the development of metacyclic trypomastigotes, the only form capable of infecting vertebrate hosts through fly bites (*Glossina* spp) which is the only vector in which *T. vivax* is able to multiply and remain in the infective phase throughout the insect's life. Outside tsetse fly areas, the parasite is carried by other hematophagous flies where transmission is noncyclical. Thus, the parasites are mechanically transmitted across vertebrate hosts, with no growth or multiplication in the insects but apart from cyclical transmission tsetse flies can also act as mechanical vectors [1]. In these cases, the fly feeds on more than one animal before repletion and

remains infective for only a short time. In South America, *T. vivax* has been disseminated by horse flies (Tabanidae) [1] and stable flies (*Stomoxys* spp.) [22]. Mechanical transmission across cows has been experimentally demonstrated for the tabanids *Cryptotylus unicolor* [12, 23], *Tabanus importunus* [24] and *Tabanus nebulosus* [25]. Although it has been proposed that the parasite might be cyclically transmitted in South America by an unknown vector other than *Glossina* [26], no evidence is available to support this hypothesis.

Mechanical transmission of African isolates of *T. vivax* was recently demonstrated in experimental conditions with tabanids [27]. These data suggest that, in Africa, mechanical and cyclical transmission co-exist in the field. However, only mechanical transmission can explain the permanent presence of *T. vivax* outside the tsetse belt. Some authors have also mentioned mosquitoes as potential vectors for *T. vivax* in Venezuela and Cuba [28]. Experimentally, *T. vivax* may also be transmitted by "syringe passage" of infective blood [29].

The degree of parasitemia in mammal hosts affects the rate of mechanical transmission of *T. vivax* [30]. A direct link between the level of parasitemia and the success of transmission can even be drawn [31]. Another factor associated with this rate is the presence of tabanids, which have larger populations in swampy areas. Cherenet *et al.* [5], Sinshaw *et al.* [6] and Otte *et al.* [32] found a significant temporal relationship between the feeding activity of tabanids and the incidence of *T. vivax*, providing evidence for the role of these insects in *T. vivax* infection. Based on the level of parasitemia and the

biting insect frequency, a mathematical model for the mechanical transmission of *T. vivax* is now under development [31].

Course of Infection, Pathogenesis and Clinical Signs:

The prepatent period of infection by *T. vivax* is variable, depending on the host and the parasite isolate. In sheep and goats, the incubation period lasts from 4 - 12 days while in bovine, it ranges from 9 - 14 days for virulent isolates and from 9 - 59 days in infections with less pathogenic isolates [1, 33]. *T. vivax* parasitemias exhibit irregular fluctuations, with some cases occurring at high levels in the morning and at lower levels in the afternoon on the same day.

At the population level, the course of the infection evolves over time. Available evidence suggesting that the level of virulence of *T. vivax* isolates is associated with the biological vector species. Thus, the isolate transmitted by *Glossina pallipides* in the central region of Kenya causes acute disease that leads to death in roughly one month in 70% of the cattle infected, while an isolate transmitted by *Glossina fuscipes* in the Kenyan province of Nyanza causes chronic infection followed by death of 100 -160 days after the initial parasitemia [1].

The Latin American strains, the infection studied under experimental conditions in 15 two year-old creole ovines [34] showed prepatent periods of 5- 20 days, with early peaks of parasitemia being very often high, accompanied by clinical signs. Depending on the cases, with the same isolate, recovery occurred naturally within 3-4 months or required treatment to avoid the death, showing the strong impact of individual host immune capacities. Relapse of the parasites in blood and clinical signs could even be induced by food restriction in sheep experimentally infected [35]. Intercurrent diseases are also known to be predisposing factors for *T. vivax* infection in bovines [36].

Once the metacyclic trypanosomes inoculated into the skin of animals, where the parasite grows for a few days and cause localized swellings (Chancres). They enter lymph nodes, then the blood stream, where they rapidly reproduce asexually by binary fission. *T. vivax* and *T. brucei* invade tissues and result in tissue damage in several organs [19, 33]. When an animal is infected with trypanosomes, antibodies against the surface coat are produced. However, trypanosomes have multiple genes, which code for different surface proteins; allowing organisms with new surface coat glycoproteins to elude the immune response. This process is referred to as

‘antigenic variation’ and results in the persistence of these organisms. The real cause that leads to the death of the animal is not fully understood. However, it is believed that the parasite releases toxic substances when it is destroyed within the circulatory system and hence damages the lining of the blood vessels. Therefore, the damage to the host does not depend on nutrients being depleted by the parasite but rather on the production of toxic substances [37].

The typical symptoms of trypanosomosis, such as cachexia, oedema, anaemia and nervous symptoms can be explained. Anaemia appears with progressing parasitaemia and there is lysis of large numbers of red blood cells resulting in a drop in PCV% [38]. The ability of trypanosomes to change their surface-coat-antigen continuously leads to the exhaustion of the antibody production by the host leading to immunosuppression [39]. In addition, there is enlargement of lymph nodes and splenomegally associated with plasma cell hyperplasia and hypergammaglobulinaemia [40]. In bovine trypanosomosis, anemia is described as normochromic-normocytic, with a tendency to be normochromic-macrocytic. Macrocytosis is due to erythropoiesis that takes place two weeks after the onset of infection, at which time, immature erythrocytes are released into the bloodstream [41, 42].

Certain African isolates of *T. vivax* can cause acute disease accompanied by hemorrhagic syndrome. Typical features of these infections include high, persistent parasitemia, fever, very pronounced anemia and generalized visceral and mucosal hemorrhage, particularly in the gastrointestinal tract. In the field, the disease affecting adult cattle can be severe enough to lead to death or miscarriage, even before diagnosis is reached and treatment can be started [9]. Severe acute anemia and thrombocytopenia are associated with the onset of parasitemia [43]. Otte *et al.* [32] showed that the disease can become subclinical in endemic areas, though it still causes sizeable losses in production. In the aparasitemic phases, trypanosomes can be found extravascularly, in lymph nodes [1], eyes (In the choroid plexus and aqueous humor) [13]. Affected animals exhibited fever, anemia, weight loss, hypoglycemia, increased serum levels of aspartate aminotransferase and nervous signs [44].

Studies undertaken on the role of *T. vivax* in sheep and goat indicated mortality and abortions in the Brazilian semiarid region, where outbreaks had been previously reported in bovines. Infected animals (25%) manifested apathy, pale mucous membranes, enlarged lymph nodes,

weakness, weight loss, opacity of the cornea, blindness and abortion [45]. The effect of trypanosomosis on reaction time and semen characteristics of 12 Zebu (Bunaji) x Friesian crossbred bulls aged between 3 and 5 years was observed for 12 weeks. Semen characteristics deteriorated progressively within the same period in infected bulls. There were highly significant and drastic decreases in sperm concentration and volume of semen and increases in sperm morphological defects. By the third week, all the infected bulls were unfit for breeding because of very poor semen characteristics. The practical implication is infertility and sterility in Zebu x Friesian crossbred bulls in trypanosome endemic areas [46]. However, the impact of *T. vivax* infection in fertility is not studied in East Africa.

According to Van Den Ingh *et al.* [47] four stages of *T. vivax* infection can be distinguished: (1) A prepatent period of about 1 week. (2) An acute or fulminating disease characterized by an almost continuous, fluctuating parasitaemia, a decrease of the number of thrombocytes, blood serotonin level, PCV and Hb, serum albumin and γ -globulin fractions, a low serum total lipid content and an initial rise with subsequent decrease of the blood bradykinin level. (3) A critical stage of about 4-5 weeks post infection during which the animal may develop a progressive parasitaemia and may die from disseminated intravascular coagulation. At necropsy microthrombi were a consistent and most significant observation. Accompanying features were oedema and haemorrhages in the various tissues, pulmonary oedema and lobular infarction, patches of necrosis in liver, spleen and adrenals and nephrotic changes in the kidneys. (4) A post-critical stage characterized by an ameliorating condition, periods with no or few trypanosomes in the peripheral blood alternating with relatively high peaks of parasitaemia. This was associated with increased blood bradykinin levels, an increase of the PCV and Hb, serum albumin and γ -globulin fractions, number of thrombocytes and blood serotonin level.

Haematological changes in Savannah brown goats experimentally infected with *T. brucei* and *T. vivax* carried out. The results indicated that the mean weight, rectal temperature, parasitemia, PCV, total plasma protein of *T. brucei* infected goats were 11.88 kg, 39.18°C, 2.40, 22.1% and 11.88 g/dl, respectively, while *T. vivax* infected goats were 12.34 kg, 39.18°C, 2.20, 23.2% and 12.34 g/dl, respectively. The values of the same parameters in the control were 14.89 kg, 38.70°C, 25.8% and 7.06 g/dl, respectively. The parasites significantly affected the haematological parameters of the animals [48].

Diagnosis and Postmortem Findings

Diagnosis: *T. vivax* infection can be diagnosed by clinical, parasitological, immunological and molecular methods.

The Clinical Picture: Pathogenic trypanosomes cause disease in all species of domesticated livestock throughout many of the tropical and subtropical regions of the world. The clinical signs of disease caused by these organisms vary according to the trypanosome species, the virulence of the particular isolate and the species of host infected. Acute disease is characterized by anaemia, weight loss, abortion and, if not treated, possibly death. Animals that survive are often infertile and of low productivity. In some instances, infected animals show no overt signs of disease but can succumb if stressed, for example, by work, pregnancy, milking or adverse environmental conditions [49]. A disease may be diagnosed on the basis of the clinical signs and symptoms. In some situations, the clinical manifestations of trypanosomosis, particularly anaemia, when taken into consideration with ecological conditions, might provide sufficient grounds for a putative diagnosis. However, the clinical signs are so varied and the ecological conditions under which trypanosomosis occur so diverse that, in terms of identifying animals with active infections, clinical diagnosis is too imprecise a procedure to use as a basis for the control of trypanosomosis and other means of diagnosis must be employed.

Parasitological Diagnosis: Examination of the blood by light microscopy is the most readily applied method for diagnosis of trypanosomosis and is a technique which can be easily applied in the field. The basic technique, i.e. examination of fresh or stained blood films, has been modified to improve diagnostic sensitivity by concentrating the blood through centrifugation in a haematocrit tube by the haematocrit centrifuge technique (HCT) or the dark ground buffy coat technique (DG) [50]. The hematocrit centrifuge technique [51] is one of the most widely used among the parasitological methods, in which motile trypanosomes can be viewed between the leukocyte layer and the plasma. The technique allows for the detection of trypanosomes six to ten days before they are detected in a fresh drop or from thick smears [52]. Other modifications suggested, but not widely applied; include the separation or removal of blood cells prior to centrifugation by miniature anion exchange chromatography or hypotonic lysis [53]. Freshly collected blood can also be inoculated into laboratory rodents

Table 1: Sensitivity of blood and lymph gland examination in the diagnosis of *Trypanosoma congolense* and *Trypanosoma vivax* infections in cattle

Trypanosome species	Number of cattle infected	Proportion of cattle found positive		
		Blood examination (%)	Lymph gland examination	Blood and lymph gland examination (%)
<i>T. congolense</i>	83	42.2	7.2	50.6
<i>T. vivax</i>	54	33.3	31.5	35.3
All infections	137	38.7	16.8	44.5

Source [55]

Table 2: Sensitivity of indirect fluorescent antibody tests and enzyme-linked immunosorbent assays in the diagnosis of trypanosomosis in infected livestock

Animals tested	Number of active infections	Proportion of animals serologically positive			
		IFAT (%)		ELISA (%)	
Cattle	<i>T. brucei</i>	5	<i>T. brucei</i>	100	
	<i>T. vivax</i>	14	<i>T. vivax</i>	85.7	
	<i>T. congolense</i>	25	<i>T. congolense</i>	88.0	
Total		47		89.4	
Cattle	<i>T. brucei</i>	2	<i>T. brucei</i>	46.3	<i>T. brucei</i> 82.1
	<i>T. vivax</i>	27	<i>T. vivax</i>	79.5	<i>T. vivax</i> 79.5
	<i>T. congolense</i>	19	<i>T. congolense</i>	66.7	<i>T. congolense</i> 82.1
Total		39		94.9	92.3
Camels	<i>T. evansi</i>	30	<i>T. evansi</i>	96.7	<i>T. evansi</i> 92.3

Source [61]

which can then be examined for periods of 30 to 60 days to determine if they have developed trypanosome infections [50].

The evaluation of some of these techniques under experimental conditions has given an indication of their detection limits in relation to the numbers of different species of trypanosomes in a blood sample. In order of decreasing sensitivity, the results were as follows: DG>HCT>thick film>thin film>wet film [50] as shown in Table 1. Varying sensitivity of the tests and the failure to detect trypanosome parasites if the number of parasites is too low, as is the case with chronic infections [54], illustrate the limitations of parasitological diagnosis and confirm the need for more reliable methods.

Immunological Techniques: Serological techniques such as immunofluorescence (IFA) and ELISA as shown in Table 2 can be used for direct and indirect diagnosis of trypanosomosis in infected hostes. Both indirect IFA refined by Katende *et al.* [56] and indirect ELISA [57, 58] are useful techniques for epidemiological investigations, especially for the determination of *T. vivax* distribution. Serological tests are available for antibody detection but they have problems related to specificity due to cross-reaction with other parasites of the same genus, especially in endemic areas where *T. evansi* infections occur [59]. Luckins [57] found that an ELISA for antibody

diagnosis in zebu cattle experimentally infected with *T. vivax*, also the assay failed to differentiate *T. brucei*, *T. vivax* and *T. congolense* infection.

The indirect fluorescent antibody test (IFAT) has been used extensively in the detection of trypanosomal antibodies in animals and humans. Antigens are usually prepared from blood smears which are fixed in acetone and then stored at a low temperature. The IFAT has proven to be both specific and sensitive in detecting trypanosomal antibodies in infected cattle [60] and camels [61]. However, cross-reactions between different trypanosome species still occur. Ashkar and Ochilo [62] found that more than 85% of cattle infected with *T. vivax* or *T. congolense* reacted with *T. brucei* antigen in the IFAT. A modification of the ELISA, currently of great interest, is based on an antigen capture assay which enables detection of circulating trypanosomal antigen in the blood of infected animals. Antibody against trypanosomal antigen is used to coat ELISA plates and any antigen present in test sera binds. The complex so formed is then incubated with the same antibody, conjugated with enzyme and visualized with a suitable substrate. The species specificity of the assay was improved following the development of monoclonal antibodies as capture antibodies that recognized antigens present in *T. brucei*, *T. vivax* and *T. congolense*. Specific circulating antigens could be detected in cattle from 8 - 14

days after infection, but within 14 days of treatment they were no longer detectable [63]. Antigen ELISA was shown to have a high diagnostic sensitivity; more than 90% and 95% in cattle and camels respectively [53, 63].

Molecular Techniques: Other more refined diagnostic techniques have been applied to study *T. vivax* such as isoenzyme analysis [64] and molecular techniques based on nucleic acids [65]. As evaluated on a South American *T. vivax* isolate [65], PCR techniques yielded excellent results, as compared with other diagnostic techniques. The principle of molecular tests is the demonstration of the occurrence of sequences of nucleotides, which are specific for a trypanosome subgenus, species or even type or strain. Nucleotides are the constituents of DNA, the molecules which constitute the genes on the chromosomes in the cell nucleus. A positive result indicates active infection with the trypanosome for which the sequences are specific, as parasite DNA will not persist for long in the host after all live parasites have been eliminated. These tests are not only suitable for detecting parasites in the mammalian host, but also in the insect vector.

A genomic clone which contained a tetramer of the 832-bp cDNA sequence was isolated and shown to be more sensitive than the monomer. Oligonucleotide primers were designed based on the nucleotide sequence of the 832-bp cDNA insert and used in amplifying DNA sequences from the blood of cattle infected with *T. vivax* isolates from West Africa, Kenya and South America [66]. The polymerase chain reaction (PCR) product of approximately 400 bp was obtained by amplification of DNA from all the isolates studied. The oligonucleotide primers also amplified DNA sequences in *T. vivax*-infected tsetse flies. Subsequently, PCR was evaluated for its capacity to detect *T. vivax* DNA in the blood of three animals experimentally infected with the parasite. *T. vivax* DNA was detectable in the blood of infected animals as early as 5 days post-infection. PCR amplification of genomic DNA of *T. vivax* is thus superior to the Ag-ELISA in the detection of *T. vivax*. More importantly, both the *T. vivax* diagnostic antigen and the gene encoding it are detectable in all the *T. vivax* isolates examined from diverse areas of Africa and South America [66].

Isolation and Purification of Blood Trypanosomes: For the improved methods of diagnosis; biochemical, biological and physical methods are available for the isolation of blood trypanosomes. One of the biochemical

methods is ion-exchange chromatography (DEAE-cellulose), described by Lanham and Godfrey [67], which is based on differences in electrical charges between erythrocytes and trypanosome cell membranes. This is not an optimal method since parasite production and viability can vary [68], however, it is the most widely used parasite separation and antigen preparation for ELISA antibody detection tests [31]. Unlike some West African stocks, the stocks of *T. vivax* isolated so far in Latin America never grow naturally in rodents. It is thus necessary to produce the antigens by cultivation in sheep or cattle, host species in which separation of parasites and blood cells on DEAE-cellulose is quite difficult and produces inconsistent results. For this reason, some authors proposed a technique based on the use of a Percoll gradient [69] or even the combined use of a gradient and DEAE-cellulose [70].

Post-Mortem Findings: The post-mortem findings in trypanosomosis can never by themselves lead to a certain diagnosis of the cause of death. There is not one single specific lesion [2]. In the acute stage there is loss of condition and anaemia, but not as severe as in chronic trypanosomosis. Microscopic examination of the blood will show that the haemopoietic system is actively trying to compensate for the loss of red cells (Regenerative changes such as anisocytosis, normoblasts, Howel-Jolly bodies and basophilic punctations). The spleen and lymph nodes are enlarged and oedematous. The liver also is enlarged and congested. The heart may be somewhat enlarged and may show a few haemorrhages on the muscle surface. There is also likely to be more fluid than normal in the chest, lungs, abdomen and pericardium. The kidneys are pale and swollen. Subcutaneous oedemas may be present particularly in horses and sheep [2].

In chronic trypanosomosis the pathological changes seen at post mortem are more striking, without being typical. The carcass is emaciated and often dehydrated. The skin may show pressure sores and ulcers, when the animal has been unable to stand up for some time. The fat reserves under the skin have been used up and the skin is closely adherent to the underlying muscles and bone. The muscles have wasted to a remarkable degree and the underlying bones are prominent. The muscles are pale because of the anaemia and the blood is watery and pale, with an increased clotting time. The heart is often enlarged and flabby because of muscle deterioration and its weaker pumping action may have contributed to circulatory disturbances and increased fluid in the tissues (Oedema). Unlike the picture seen in acute trypanosomosis, the lymph nodes are mostly normal or

even hard, dry and reduced in size. The spleen is also normal in size or contracted with a drier pulp than normally seen [2].

Epidemiology: The epidemiology of trypanosomosis is dependent on the interactions between the parasite, vector and host factors. However, in non tsetse-transmitted trypanosomosis (*T. vivax* and *T. evansi*) the epidemiology is also influenced by many factors. There may be seasonal outbreaks, where the populations of biting flies (Tabanids, stable flies, etc.) are influenced by important seasonal climatic differences. The chronic disease sometimes becomes more clinically apparent during the dry season, when immunodepressive factors such the poor nutritional state of the animal. The epidemiology is also greatly influenced by host preferences and daily behaviour patterns of the various local species of tabanids and other biting flies.

In Africa, several species of ungulates are hosts for *T. vivax*, including not only domestic animals such as bovines, ovines and caprines, but also equids, camelids, to which the parasite is pathogenic and wild animals, such as several species of antelope, to which it is innocuous [1]. In tropical Africa, *T. vivax* is found in an extensive area that is a habitat for flies of the genus *Glossina*. Outside the tsetse area, the parasite is also found in most of the western, eastern and central regions of Africa [19]. It is generally thought that *T. vivax* was introduced into the New World at around 1830, when Zebu cattle from Senegal were exported to French Guiana. However, it is not possible to identify dates and means of introduction in these countries [35].

In common with the other Salivarian trypanosomes, *T. vivax* is transmitted by a diverse range of *Glossina* spp. and thus is primarily found within the tsetse belt of sub-Saharan Africa. *T. vivax* may also be efficiently transmitted mechanically by a number of biting flies, [5, 6, 8, 27, 71] which has allowed it to become established outside the tsetse belt of Africa and in large parts of South America. The reported prevalence of *T. vivax* shows considerable variation, with geography, abundance of tsetse or biting flies and host species all important variables. Within the tsetse belt overall *T. vivax* prevalence is typically reported within the 5-15% range and will often account for up to half of total trypanosome prevalence [72]. Outside of the tsetse belt *T. vivax* prevalence is typically lower, in the range of 2-10% [6, 71] and dependent upon local and seasonal variation in fly abundance. When tsetse flies are absent *T. vivax* typically accounts for all trypanosome infections in African livestock [5, 71].

True prevalence rates may however be much higher than these commonly reported values due to the limited sensitivity of the microscopy based techniques typically utilised [71]. Faye *et al.* [73] observed an approximately seven times higher prevalence of bovine trypanosomosis when comparing PCR-based identification with the traditional microscopy technique in an analysis of herds in regions of high and low tsetse challenge in The Gambia. Similarly, Pinchbeck *et al.* [74] recently reported a significantly higher detection of prevalence in clinical equine samples from The Gambia when comparing PCR detection with microscopy, reporting a *T. vivax* prevalence of 87% from PCR identification, compared with a total trypanosome prevalence (*T. vivax*, *T. congolense* and *T. brucei*) of only 18% with microscopy. In a study conducted in Chad to estimate the prevalence and the incidence of *T. vivax* infection in herds of cattle from tsetse free areas of Lake Chad region indicated that the prevalence was 1.6% using BCT and 42.3% with indirect ELISA [71].

The infection can be seen in Africa at some distance from the edges of tsetse belts along the White Nile from Malakal in the southern Sudan up into the semi-desert of Khartoum Province, hundreds of kilometres from any tsetse belt. A similar situation has been reported in Ethiopia, where *T. vivax* is commonly found in highlands too cold for tsetse survival. But the most remarkable fact is that *T. vivax* has been able to establish itself in the South America, in the absence of tsetse. These American strains of *T. vivax* are thoroughly adapted to mechanical transmission and all attempts to transmit them biologically through tsetse have failed. In the past, *T. vivax* has also been present on the Indian Ocean island of Mauritius, without tsetse, but has been eradicated there. There are also indications that *T. vivax* may sometimes persist at a low level, because of mechanical transmission, after tsetse flies have been eradicated from an area [2]. The unregulated movement of infected animals across national and international borders is probably the principal way that the parasite spreads to new areas. Once introduced into an area, however, the method of subsequent transmission is not clear [8].

In order to fully understand and manage the disease caused by *T. vivax* and other trypanosomes, a better understanding of the parasite's basic biology and population dynamics, in particular the mode of reproduction is essential. For instance, the exchange of genetic material will allow predictions of its ability to adapt to environmental changes and to develop and spread traits, such as drug resistance, that are important for understanding both the epidemiology of the disease

and its control. There have been few population studies to assess the diversity and population dynamics of *T. vivax* [75].

Host Immunity to Trypanosomes

Immune Response: The antigen–antibody reaction triggers immune response mechanisms that promote host resistance to *T. vivax* infection. One such mechanism involves the formation of a lysis complex that annihilates the parasite by activating the complement system. Additional mechanisms include phagocytosis, rendered more efficient by antibody opsonization and antibody-dependent cytotoxicity [76].

As a result of their co-evolution, trypanosomes are often nonpathogenic in wild animals [77]. Similarly, some breeds of cattle remain relatively healthy and productive in areas where tsetse-transmitted trypanosomosis occurs [38]. N'Dama cattle are more resistant to *T. congolense* [78], *T. brucei* [38] and the West African isolates of *T. vivax* than Boran cattle. However, both breeds of cattle are highly susceptible to infection with hemorrhagic strains of *T. vivax* [79].

Nonimmunological factors may have a role in the destruction of host erythrocytes associated with *T. vivax*, such as sialidase, a neuraminidase that hydrolyzes sialic acid *in vitro* [80]. Sialic acid is an important component of the erythrocyte surface membrane. Its removal from the erythrocyte surface is normally an age-dependent process and leads to removal of aged cells through phagocytosis. Esievo *et al.* [80] reported a decline in the amount of sialic acid on the erythrocyte surface in cattle infected with *T. vivax* leads to anemia because of the process described above. Also, serum levels of complement in *T. vivax* and *T. congolense* susceptible cattle are decreased, which results in impaired phagocytosis and impaired immunoregulatory activity of the complement. Complement and complement receptors on the surface of follicular dendritic cells play a role in the production of IgG and the formation of memory cells [81].

Immunosuppression: Host immunosuppression is a well-known feature of bovine and human trypanosomosis [39, 82]. Immunosuppression was first identified based on increased susceptibility of trypanosome infected hosts to secondary infections. This is a relevant phenomenon, as cattle infected in the field often die of opportunistic infections. Disease associated suppression also affects host immune response to trypanosomes. In some cases, suppression is specific, involving only the host response to the parasite; in others, generalized suppression occurs in the infected animals, marked by a decrease in T cell

proliferation and cytokine production [83]. Several studies have been conducted on the humoral response to parasite antigens. Williams *et al.* [84] and Taylor *et al.* [85] found that trypanosome susceptible cattle produce lower levels of IgG against trypanosome specific antigens than do tolerant cattle.

Immunity at the Population Level: The transmission of several *Trypanosoma* species (*T. vivax*, *T. congolense* and *T. brucei*) by tsetse flies in which the parasites multiply and undergo genetic recombination, leads to a high incidence of infections with high infective doses and a high genetic diversity of parasites. In such conditions, a large portion of the cattle population is unable to control the infection [86]. For comparison of these epidemiological features, Figure 5 shows a model of trends in parasitological and serological prevalence of *T. vivax* in cattle populations that are exposed to mechanical vectors (Figure 5A) compared to cyclical vectors (Figure 5B).

In areas where transmission of *T. vivax* is strictly mechanical, such as in Latin America, bovine trypanosomosis occurs in the form of periodic multiple epizootic outbreaks against a subclinical enzootic background. Because mechanical transmission is unpredictable, the epidemiological situations are unstable. Following an 'epizootic wave' (Figure 5A, phase 1), infections are gradually eliminated by self-curing and treatment. During this period, which can be referred to as the 'inter-epizootic period' (Figure 5A, phase 2), concomitant immunity becomes established and maintains very low parasitemia. After several years, most of the population becomes susceptible and when other circumstances arises another 'epizootic outbreak' (Figure 5A phase 3) is triggered. This phenomenon, which we can refer to as 'an epidemiological seesaw' is typical of mechanically transmitted bovine trypanosomosis and has been observed in several South American countries [34]. This alternation between 'epizootic clinical' and 'inter-epizootic subclinical' phases is characterized by long silent periods during which the parasite is not visible followed by very widespread clinical explosions [35].

In Africa where the disease is cyclically transmitted by *Glossina*, these cycles do not arise and trypanosomosis is a permanent blight that is constantly detectable. Although the incidence of infection and parasitological, serological and clinical prevalence varies on a seasonal basis (Figure 5B), there are never any wholly silent periods because *Glossina* are permanent carriers of several of the parasite species. Mechanical transmission also occurs in addition to cyclical

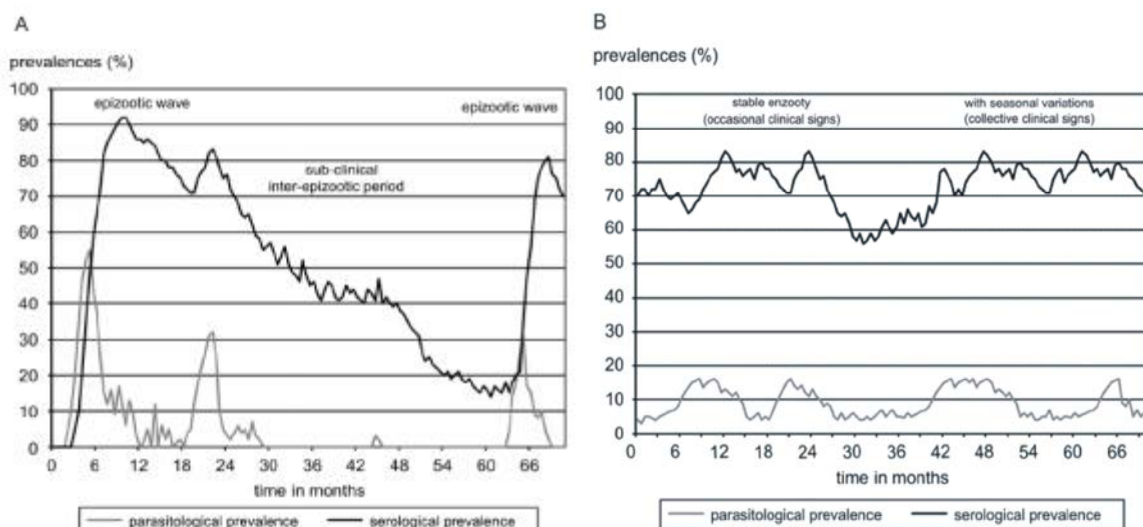


Fig. 5: Model of trends in parasitological and serological prevalence of *T. vivax* in cattle populations that are exposed to (A) mechanical and (B) cyclical vectors, Source [8]

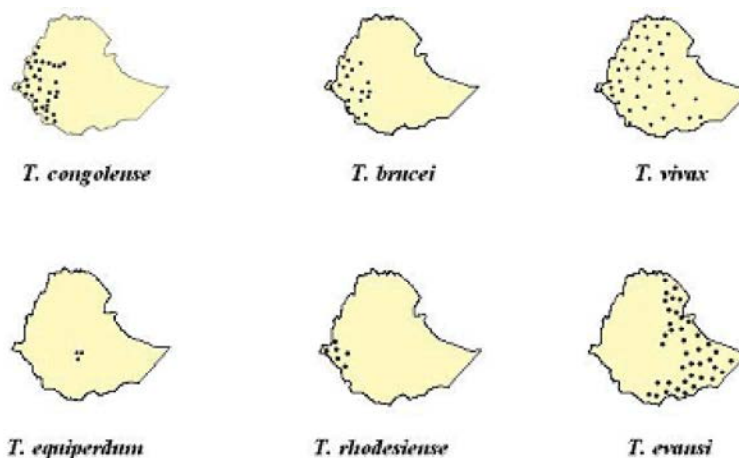


Fig. 6: Distribution of pathogenic trypanosomes in Ethiopia, Source [3]

transmission and contributes to the intensification of *T. vivax*, allowing for the maintenance and a high prevalence of infection for this particular species [35].

Trypanosomosis in Ethiopia: Trypanosomosis is prevalent in two main regions of Ethiopia i.e. the northwest and the southwest regions [3]. In these regions tsetse transmitted animal trypanosomosis is a major constraint to utilization of the large land resources. The northwest region of Ethiopia is particularly affected by both tsetse and non-tsetse transmitted trypanosomosis [4-6]. Six species of trypanosomes are recorded in Ethiopia and the most important trypanosomes species, in terms of economic loss in domestic livestock are: *T. congolense*, *T. vivax* and *T. brucei* as shown in Figure 6. The closely related *T. brucei* subspecies, *T. b. rhodesiense* causes

human sleeping sickness. The other *trypanosoma* species of economic importance are *T. evansi* of camels and *T. equiperdum* of horses [3].

The most prevalent trypanosome species in tsetse-infested areas of Ethiopia are *T. congolense* and *T. vivax*. Abebe and Jobre [4] reported an infection rate of 58.5% for *T. congolense*, 31.2% for *T. vivax* and 3.5% for *T. brucei* in southwest Ethiopia. In the same report 8.7% prevalence rate was recorded in the highlands (Tsetse fly free areas) of which 99% is due to *T. vivax*. The prevalence of bovine trypanosomosis in tsetse infested areas of northwest Ethiopia in late rainy and dry seasons were 17.07% and 12.35% respectively [87]. The prevalence of bovine trypanosomosis in tsetse-infested and tsetse-free areas of northwest Ethiopia were 13.5% of wet blood film and 15.6% of thin blood film while PCR detected 18%

trypanosome infections. The dominant species was *T. vivax*, followed by *T. congolense* and *T. brucei*. The monthly prevalence of infection was correlated with the density of biting flies suggesting their important role in transmission of trypanosomosis. In this study a total of 5652 tsetse and other biting flies were captured. PCR amplification analyses for trypanosome identification were carried out on 3751 flies, with primer sets specific for *Trypanosoma (Duttonella) vivax*, *T. (Nannomonas) congolense* and *T. (Trypanozoon) brucei*. Of 3751 flies 18.64% were positive in PCR analysis with 12.13% from tsetse-free areas and 21.29% from tsetse-infested areas. Comparing within the type of flies, out of 1314 tsetse flies (*Glossina m. submorsitans* and *Glossina tachinoides*) 27.85% were positive and out of 2437 other biting flies 13.66% were found positive [5].

Apart from the cyclical transmission of trypanosomosis by the *Glossina* spp. it is highly considered that mechanical transmission is a potential threat to livestock productivity in Ethiopia [4]. According to Sinshaw *et al.* [6] epidemiological investigation of mechanically transmitted trypanosomosis in three districts (Bahir Dar Zuria, Dembia and Fogera) bordering Lake Tana of northwest Ethiopia indicated that the prevalence of trypanosomosis in cattle was 6.1%. Among small ruminants only one sheep 0.8% (1/122) and one goat 0.15% (1/676) were found positive for *Trypanosoma* species and none of the equines were positive. All the trypanosomes found belong to a single species of *T. vivax*. In the same study a total of 71,273 flies were caught of which 69.2% belong to Stomoxys, 22.3% to non-biting Muscidae, 6.6% to horse flies and 1.9% to Chrysops and there was no tsetse fly.

In Ethiopia currently the most widely used trypanocidal drugs for *T. congolense* and *T. vivax* infection are isometamidium chloride and diminazine aceturate. The occurrence of drug resistant trypanosome across Ethiopia is not well known. Trypanocidal resistance particularly against *T. congolense* infection is reported in the Ghibe Valley. Recent investigation in Metekel district of northwestern Ethiopia, western Ethiopia and in Dembecha district of northwest Ethiopia indicates the occurrence of drug resistant *T. congolense* infections [88-90] respectively. But the status of resistant isolates of *T. vivax* against the available trypanocidal drugs is not performed compared to *T. congolense*.

Treatment and Control: Control of the disease should combine restricted movement of diseased animals, treatment of *T. vivax* infected animals, epidemiological monitoring of the distribution and severity of the disease and vector control [12].

Host Treatment: The application of antitrypanosomal drugs has been the most widely practiced means of controlling trypanosomosis in domestic livestock since the early 1950s, either as curative or prophylactic drugs. A programme to eradicate tsetse flies will be complex, take many years and possibly cost some US\$ 20 billion [10]. Thus, control of trypanosomosis will depend in the foreseeable future on the use of the existing trypanocidal drugs. The challenge, therefore, remains to make optimal use of the three relatively old compounds until new methods of treatment emerge. The three antitrypanosomal compounds upon which treatment and prophylaxis of cattle trypanosomosis currently depends are isometamidium chloride, homidium chloride or bromide and diminazene aceturate (Table 3).

The satisfactory treatment of trypanosomosis is largely determined by the plain of nutrition, the amount of exercise during convalescence and the duration of the disease. Well-rested and well-fed animals recover more rapidly after trypanocidal therapy than do undernourished animals that have to trek long distances to reach pastures. The development of trypanosome resistance to trypanocides is a continuous threat to their sustainable use in the control of trypanosomosis [16]. Even in areas where resistance to trypanocides has not yet been demonstrated, the probability of its development should influence the selection of an appropriate control strategy [29].

Vector Control: In the absence of vaccines and effective, affordable drugs, African trypanosomosis control relies heavily on vector control with eventual impacts ranging from reduction of fly populations to total eradication. Targets and traps have been effective in controlling populations locally and have been used extensively in agricultural settings and considerable success has been achieved by directly applying insecticides on animals (Pour-on) [91]. Discriminative spraying of just the resting sites of tsetse would reduce costs, cause less environmental pollution and would be easier to carry out as only a small percentage of the total tsetse habitat would be sprayed. However, the technique is labor intensive, demands high level of supervision and has effects on non-target organisms [92].

Tsetse is visually attracted to specially designed traps or targets. This attraction may be augmented by the use of olfactory attractants [93]. Despite successful field trials however, livestock farmers and national governments in Africa have been slow to embrace traps and targets as a means of tsetse control. The reason for this was difficulties associated with development and maintenance of traps and targets over large and often in

Table 3: Commercial trypanocides for the treatment and control of trypanosomosis

Generic name	Trade names	Solution for use	Dosage rate	Route	Remarks
Suramin	Naganol	10%	10 mg/kg (1 ml/10 kg)	IV	Mainly used against <i>T. evansi</i> in camels
Diminazene aceturate	Berenil, Ganaseg, Trypazen, Veriben	7%	3.5-7 mg/kg (1-2 ml/20 kg)	IM	Mainly used in cattle and small ruminants
Homidium bromide	Ethidium bromide	2.5%	1 mg/kg (1 ml/25 kg)	IM	Mainly used in cattle and small ruminants. Should be dissolved in hot water. Potentially carcinogenic
Homidium chloride	Ethidium C, Novidium	2.5%	1 mg/kg (1 ml/25 kg)	IM	See above, but soluble in cold water
Quinapyramine methyl sulphate	Antrycide, Trypacide, Noroquin, Quintrycide	10%	5 mg/kg (1 ml/20 kg)	SC	Now mainly used against <i>T. evansi</i> and <i>T. brucei</i> in camels and horses
Melcy	Cymelarsan	0.5%	0.25-0.5 mg/kg (1-2 ml/20 kg)	IM or SC	Registered only for use against <i>T. evansi</i> in camels
Isometamidium chloride	Samorin, Trypamidium	1% 2%	0.25-0.5 mg/kg (1.25-2.5 ml/50 kg) 1.0 mg/kg (2.5 ml/50 kg)	IM	Used mainly in cattle, as a curative at lower rates, as a prophylactic at higher rates. Also contains homidium and is therefore to be considered as potentially carcinogenic as well

Source [2]

accessible areas and the cost of odor attractants like acetone and insecticides [93]. Insecticide treated cattle as pour-on offer numerous advantages over-baited traps and targets. Cattle are used as moving targets and hence no cost on odor baits. The technique was field tested and found successful in a number of African countries such as Ethiopia [91, 94].

An alternative approach is the sterile insect technique was applied in large scale tsetse eradication programs in some parts of Africa including Burkina Faso, Tanzania and Zanzibar [95]. Perhaps the most notable example of the success of SIT, after tsetse population suppression with targets and pour-on, is in Zanzibar where *G. austeni* has been eradicated from the Island [96]. Despite such efforts and successes in vector control in tsetse infested areas of Africa, little attention has been paid to vectors of trypanosomes outside the tsetse zone (Such as for *T. vivax*). In addition, the absence of a clearly defined vector makes it practically impossible to incorporate targeted vector reduction methods for control of South American *T. vivax* in the way that they have been used in Africa for tsetse-transmitted trypanosomes [97]. The antigenic complexity of trypanosomes has thwarted attempts to develop a vaccine [98]. Although potential immunological targets within the parasite have been identified, no vaccine will be commercially available in the near future and the greatest hope for the immunological control of animal trypanosomosis lies in the exploitation of trypanotolerant breeds of livestock [99].

Trypanocidal Drug Resistance

Current Situation: Drug resistance is defined as a loss of sensitivity by a strain of an organism to a compound to

which it had been previously susceptible [100]. It implies failure of treatment or prevention and if no other active drugs are available the animal has to rely on its immune defences alone to combat the disease [2].

The problems of drug resistance have been reported from 17 countries in sub-Saharan Africa including Ethiopia [16]. This is probably an underestimation of the true situation, because in several countries surveys for resistance have not yet been carried out or cases of resistance have not been published. In eight of the 17 countries, multiple resistances have been reported. Most of the currently available information on drug resistance, however, is derived from limited numbers of case reports and does not give any indication of the prevalence of drug resistance in a region or a country as systematic surveys have not been conducted. There is an urgent need for surveys in which representative numbers of trypanosome isolates are examined for drug resistance particularly out of tsetse belt areas where mechanical transmitted trypanosomosis is prevalent and information is limited [16].

The control of New World *T. vivax* relies heavily on drug therapy, principally based on diminazene aceturate and ISMM used in some areas. Such an indiscriminate use of drugs is thought to be a major factor in encouraging the appearance of drug-resistant populations and resistance to diminazene aceturate has been reported in Colombia and French Guiana [101]. Furthermore, unrestricted animal movements are likely to add to the problem by spreading resistant populations. In spite of this, little information is available on the sensitivity of mechanically transmitted isolates of trypanosomes to trypanocides.

Detection of Trypanocidal Drug Resistance: Several methods have been described to identify drug resistance in trypanosomes as reviewed by Delespaux *et al.* [16]. At present, three types of techniques are commonly used to identify drug resistance: tests in ruminants; tests in mice; and *in vitro* assays. None of these is an ideal test and other tests are still in the phase of development or validation.

Tests in Ruminants: Tests in ruminants provide direct information from studies in ruminants using recommended doses of trypanocidal drugs. The tests commonly consist of infecting a group of cattle or small ruminants with the isolate under investigation and later, when the animals are parasitaemic, treating them with various levels of trypanocide. The animals are then regularly monitored over a prolonged period (Up to 100 days) to determine the effective dose (ED), i.e. the dose that clears the parasites from the circulation and the curative dose (CD), i.e. the dose that provides a permanent cure [102]. Cattle or small ruminants in these studies must be kept in fly-proof accommodation or in a non-tsetse area in order to eliminate the risk of re-infection during the study. A useful indication of the level of resistance can be obtained from studies in ruminants by recording the length of time between treatment and the detection of break through populations of trypanosomes. Most trypanosome isolates of cattle are able to grow in these hosts and that the data obtained are directly applicable to the field.

In vitro Assays: Further progress has been made in the field of *in vitro* assays [103]. The advantage of this technique is that large number of isolates can be examined; tests with metacyclic trypanosomes correlate well with field observations. The disadvantages of this technique are: *in vitro* cultivation of blood stream forms is only possible using pre-adapted lines and not using isolates directly from naturally infected animals in addition it is expensive; require good laboratory and well-trained staff.

Trypanocidal Drug ELISA: The use of trypanocidal drug ELISA in combination with parasite detection tests has given promising results for the detection of resistant trypanosomes. The test is both sensitive, detecting subnanogramme concentrations and specific. It allows the monitoring of drug levels over extended periods and the evaluation of factors influencing drug disappearance rates from the plasma. One interesting finding has been that the drug disappears more rapidly in animals challenged and

becoming infected with drug-resistant trypanosome isolates than in those challenged but protected against infection with sensitive trypanosomes [104]. Observations showed that the presence of trypanosomes in animals with an ISMM concentration of > 0.4 ng/ml suggests resistance; the higher the drug level detected the greater the degree of resistance that could be inferred [105]. It is not yet possible to draw firm conclusions on the sensitivity or resistance of a trypanosome population at the level of individual animal. The ELISA should, however, some indications of the resistance situation at the level of the herd.

Field Methods for Rapid Assessment of Trypanocidal Drug Resistance: To overcome constraints associated with laboratory tests, field methods to assess resistance of trypanosomes to trypanocides in cattle herds under natural tsetse challenge, have been proposed. Rowlands *et al.* [106] developed a model to distinguish new and recurrent infections in order to determine if the high infection rates observed in cattle in Ghibe valley, south west Ethiopia following treatment of *T. congolense* infections with Diminazine aceturate were due to the tsetse challenge or if they were instead due to relapse of infections following treatment. An infection was defined as a new infection if it was preceded by two previous months in which monthly collected samples had a PCV $>26\%$ and were not detected with trypanosomes. Eisler *et al.* [107] have developed a method for the assessment of trypanosomosis risk and the level and prevalence of resistance to ISMM, utilizing cattle populations under natural challenge in the field. This protocol compares new trypanosome infections in a group of cattle treated with ISMM to untreated group. The rate at which new infections occur in the two groups is assessed by a comparison of their survival curves over an 8-12 weeks period. This provides a rapid and accurate assessment of ISMM resistance and the impact of drug use relative to no treatment [107].

Molecular Techniques: PCR has also been used to monitor the efficacy of diminazene aceturate treatment in cattle experimentally infected with *Trypanosoma brucei* [108]. Under natural challenge, PCR and DNA probe hybridization were used to confirm the effectiveness of isometamidium chloride prophylaxis in cattle infected with *T. brucei* and *T. vivax* populations [108]. Amplified Fragment Length Polymorphism (AFLP) was used recently by Delespaux *et al.* [109] to compare the genome of 2 isogenic clones of *T. congolense* in order to search for

mutations that might be correlated with the resistance of these trypanosomes to isometamidium. The correlation between the single dose mouse test and the PCR-RFLP test was consistent in 30 of the 35 tested isolates. Preliminary results show also that Single Strand Conformation Polymorphism (SSCP) allows the detection of *T. congolense* resistance to diminazene [110]. *T. congolense* putative gene (TcoAT1) presenting a high similarity with the adenosine transporter 1 gene (TbAT1) of *T. brucei* and coding a putative P2-like nucleoside transporter was screened by SSCP for point mutations possibly linked to changes in sensitivity to diminazene. Using the commonly accepted criterion for sensitivity to diminazene, being a CD80 of 20 mg/kg in the mouse test, there was a correlation of 84.6% (22 out of 26 isolates). Although PCR, RFLP and SSCP need a well equipped laboratory, they could provide a rapid and convenient tool, suitable for large-scale surveys of drug-resistant trypanosomes in livestock.

Economic Impact: African trypanosomes cause an important impact on human welfare, resulting in income loss in very resource-poor settings, with an estimated cost of US \$1.3 billion per year [111]. Despite the considerable impact on animal health and the subsequent downstream effects on human populations, many aspects of the disease remain poorly understood. In Africa, losses caused by bovine trypanosomosis have been estimated at roughly 5 million \$ annually if declines in beef production are taken into account, not to mention losses in milk production and secondary products, such as leather [43]. According to Budd [10] African farmers spend 35 million US \$ per year on trypanocidal drugs to protect and cure their cattle. Losses in meat production, milk yield and traction power are estimated to cost approximately US \$ 500 million annually. A pondered evaluation extrapolated for the total tsetse-infested lands values the total losses, in terms of agricultural Gross Domestic Product, at US \$ 4.75 billion per year [11].

The impact of bovine trypanosomosis in Latin America has not been satisfactorily estimated, although it is thought to rank third among parasitic diseases in economic importance, after tickborne diseases and fasciolosis in Colombia [32, 112, 113] suggested that even inapparent losses from subclinical infections might be sizeable. The economic impact of this disease has been estimated for the Brazilian Pantanal and Bolivian lowlands of South America, where with a cattle population estimated at 11 million, approximate losses due to *T. vivax* infection could exceed \$160 million [114, 115]. No financial estimates are available for other countries in the region.

The inapparent losses of subclinical infections by *T. vivax* may be considerable and the same certainly applies to mechanically transmitted trypanosomosis in Africa; further economic studies are necessary in order to obtain reliable figures.

In addition to the mortality that occurs in peracute and acute cases of trypanosomosis, it also leads to reproductive disorders [116]. General reproductive failure and poor lactation performance of *Bos taurus* cattle introduced into high tsetse challenge areas was reported by Anene *et al.* [117]. Disruption of oestrus cycles has been reported in trypanosome-infected Boran cows with most infected ones becoming acyclic [118]. Long calving intervals and abortions or stillbirths are common in trypanosome infected cattle in the field. Other production losses due to trypanosomosis include neonatal mortality and poor growth rates. It has been shown that infected bulls were unfit for breeding due to poor quality of semen and lack of libido [119].

Genetic Diversity: Genetic diversity refers to any variation in the nucleotides, genes, chromosomes, or whole genomes of organisms. Genetic diversity at its most elementary level is represented by differences in the sequences of nucleotides (Adenine, cytosine, guanine and thymine) that form the DNA (Deoxyribonucleic acid) within the cells of the organism. The DNA is contained in the chromosomes present within the cell; some chromosomes are contained within specific organelles in the cell (For example, the chromosomes of mitochondria and chloroplast). Nucleotide variation is measured for discrete sections of the chromosomes, called genes. Thus, each gene comprises a hereditary section of DNA that occupies a specific place of the chromosome and controls a particular characteristic of an organism [84].

Isoenzymes: Isozymes (Also known as isoenzymes) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. These enzymes usually display different kinetic parameters or different regulatory properties. The existence of isozymes permits the fine-tuning of metabolism to meet the particular needs of a given tissue or developmental stage (For example lactate dehydrogenase (LDH)). In many cases, they are coded for by homologous genes that have diverged over time. Although, strictly speaking, allozymes represent enzymes from different alleles of the same gene and isozymes represent enzymes from different genes that process or catalyse the same reaction, the two words are usually used interchangeably [120].

The first clear evidence for genetically distinct form of *T. vivax* was provided by Allospp and Newton [121] showed rodent infectivity and the ability to induce a haemorrhagic syndrome could be correlated with isoenzyme profile. Later work has associated some isoenzyme patterns with geographical origin. Isoenzymatic and karyotype studies carried out in Colombia by Dirie *et al.* [7] showed strong relationships with West African strains; however, immunolysis tests revealed full cross protection amongst Colombian stocks, but only partial protection with West African stocks.

Karyotype and Kinetoplast DNA: *T. vivax* has a similar molecular karyotype to other salivarian trypanosomes, with chromosomal DNA ranging in size from approximately 50 to 6000 kb. However unlike other salivarian trypanosomes which have an estimated 100 mini-chromosomes of 50-100 kb, *T. vivax* has one or two mini-chromosomes which are characterized by the presence of a highly repetitive guanine/cytosine-rich satellite DNA of 177 bp in size [122]. *T. vivax* kinetoplast DNA minicircles are approximately half the size of other salivarian trypanosomes while the relevant amount of maxicircle DNA is high at least twice that of *T. brucei*.

DNA Sequence Analysis and Molecular Characterization: The study of genetic diversity in *T. vivax* has been hindered by the difficulty of growing most *T. vivax* strains in laboratory rodents or culture, but wider sampling is now possible through PCR-based methods, for example Craig *et al.* [123] investigated the population structure of *T. vivax* in The Gambia by microsatellite analysis of blood samples from infected livestock. While these West African field samples showed a clonal population structure, the single Kenyan sample used for comparison was divergent with unique alleles at half the microsatellite loci examined. This finding is echoed by several other studies suggesting genetic divergence of East and West African strains of *T. vivax* and significant diversity within *T. vivax* in East Africa. Firstly, isoenzyme and DNA fingerprinting studies showed that West African, Ugandan and South American isolates were similar, Kenyan isolates, including some known to cause the haemorrhagic syndrome, were very divergent [7, 124]. Secondly, West African, Ugandan and South American *T. vivax* isolates hybridized with the 180 bp satellite DNA repeat from a Nigerian *T. vivax* [122], it did not hybridize with four isolates of *T. vivax* from Kenya [64]. It has now become apparent from molecular epidemiological studies that the PCR test based on this sequence fails to pick up

many *T. vivax* infections, particularly in East Africa [125]. In Tanzania, further investigation of some of the non-identified trypanosomes from infected tsetse proboscides revealed a *T. vivax* related trypanosome with 86% sequence identity in a 300 bp fragment of the 18S rRNA gene to the Nigerian reference isolate, *T. vivax* ILDat 1.2 (Y486) [126]. Cortez *et al.* [126] found that the Kenyan *T. vivax* strain, IL3905, was distinct from West African and South American *T. vivax* by comparison of 18S rRNA (V7-V8 region), ITS and 5S rRNA gene sequences.

Molecular based phylogenetic analysis of *T. vivax* based on 18S SSU and 28S LSU RNA sequences from a single isolate of *T. vivax* lineage shows a greatly elevated substitution rate within its rRNA sequences. Analysis suggest that it may have undergone 3 to 8 times as many substitutions as the lineage of many non-salivarian trypanosomes [75] and may be evolving more than twice as fast as other salivarian trypanosomes. The well supported early separation of the *T. vivax* lineage is consistent with view that *T. vivax* represents the most ancient of the salivarian [128] and that *T. vivax* represents an early stage of adaptation to transmission by tsetse flies [129]. *T. v. veinni* found in South America is morphologically identical to African *T. vivax* and its inability to undergo cyclical development in tsetse appears to be the only clear behavioural attribute of the subspecies, but, no investigations have been carried out to determine the basis of this trait.

The sequence of the spliced-leader gene repeat of a Brazilian *T. vivax* stock from cattle showed high similarity to sequences of West African *T. vivax* in both intron and intergenic sequences. Cortez *et al.* [127] compared South American isolates (Brazil and Venezuela) with West and East African *T. vivax* isolates. Phylogeny using ribosomal sequences positioned all *T. vivax* isolates tightly together on the periphery of the clade containing all Salivarian trypanosomes. The same branching of isolates within the *T. vivax* clade was observed in all inferred phylogenies using different data sets of sequences. *T. vivax* from Brazil, Venezuela and West Africa (Nigeria), corroborating the West African origin of South American *T. vivax*, whereas a large genetic distance separated these isolates from the East African isolate (Kenya) analyzed. Genetic characterization by PCR using random oligonucleotides as primers (RAPD) and PCR based on repeated extragenic palindromic sequences (REP-PCR) and repeated intergenic sequences (ERIC-PCR) revealed polymorphisms among the isolates, but with coefficients of similarity of 0.69, indicating high genetic proximity. From the knowledge acquired to date, it appears that the genetic diversity of

Latin American *T. vivax* isolates is much lower than that of African strains [35], most probably due to the lack of genetic recombination, which occurs only in the intermediate tsetse host [130]. These observations help explain the ability of South American cattle populations to temporarily control the infection while such control is not possible in Africa.

Evidence for mating in some of the related trypanosome species, *T. brucei*, *T. congolense* and *T. cruzi* is reported but very little work has been carried out to *T. vivax*. Understanding whether mating occurs will provide insight into the dynamics of trait inheritance, for example the spread of drug resistance, as well as examining the origins of meiosis in the order Kinetoplastida. In order to address whether mating occurs, a sympatric field population of *T. vivax* collected from livestock in the Gambia, using microsatellite markers developed for this species the analysis identified a clonal population structure showing significant linkage disequilibrium, homozygote deficits and disagreement with Hardy–Weinberg predictions at six microsatellite loci, indicative of a lack of mating in this population of *T. vivax* [123].

The sequence of appearance of specific lytic activity against more than 20 variable antigen types (VATs) of *T. vivax* in the serum of 27 animals belonging to 5 species has been examined to determine the sequence of antigenic variation. The sequence of antigenic variation was similar in all host species, with some VATs consistently eliciting response more rapidly than others. There was very little evidence for differences in appearance of VATs between host species; the only clear example was one VAT which apparently did not develop in one host species. The sequence of antigenic variation in *T. vivax* seems to be determined by the parasite rather than the host species [131]. One of the variant surface glycoproteins (VSGs) of a West African stock of *T. vivax* was identified, purified and partially characterized by the use of a combination of highly resolving techniques to maximize information from the relatively small amount of parasite material available. The molecular weight of the isolated protein (46,000) is smaller than that of VSGs from other species. The small size of the *T. vivax* VSG may have a bearing on the functional and evolutionary relationships of variant antigens in trypanosomes [20].

Recent survey on trypanosomes carried out in Tanzania from DNA samples of infected proboscides of *Glossina pallidipes* and *G. swynnertoni* using fluorescent fragment length barcoding (FFLB), which discriminates species by size polymorphisms in multiple regions of the ribosomal RNA locus. FFLB identified the trypanosomes

in 65 of 105 (61.9%) infected proboscides, revealing 9 mixed infections. Of 7 different FFLB profiles, 2 were similar but not identical to reference West African *T. vivax*; 5 other profiles belonged to known species also identified in fly midguts. Phylogenetic analysis of the glycosomal glyceraldehyde phosphate dehydrogenase gene revealed that the Tanzanian *T. vivax* samples fall into 2 distinct groups, both outside the main clade of African and South American *T. vivax*. These new *T. vivax* genotypes were common and widespread in tsetse in Tanzania [132].

CONCLUSION

Trypanosomosis caused by *T. vivax* causes an acute to chronic infections in most domestic animals affecting the productivity of animals and the overall agricultural activities in the tropical countries. It is exceptionally important from other species of trypanosomes due to the transmission ability of *T. vivax* involving both cyclical and mechanical ways so that the disease is established itself out of tsetse belt areas of Africa and in South America. In Africa the attention given for non-tsetse transmitted trypanosomosis is limited for example controlling trypanosomosis is focused on tsetse transmitted trypanosomosis which might not be effective since *T. vivax* can exist as a potential problem in the absence of tsetse. The control of the disease is based on treatment with an integrated approach; however, trypanocidal drugs have been in challenge because of the development of drug resistance. The available information on the pathogenic impact, the web of transmission as well as the occurrence and distribution trypanocidal drug resistance mainly on *T. vivax* is limited and this is generally indispensable in Ethiopia. Therefore based on this remark the following recommendations are forwarded.

- The consequence of the wide distribution of *T. vivax* out of tsetse infested region and within tsetse belt areas is not well known which seeks further research for the control of the disease.
- In Ethiopia studies should be conducted on the impact of *T. vivax* such as pathogenicity, trypanocidal drug resistance and at large its economic importance.

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